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EXAMINER	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/685,837	SEIBLER ET AL.	
	Examiner	Art Unit	
	ANOOP SINGH	1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *1/28/2009*.

2a)☒ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,5,6,8-12,15-24,26,27 and 30-38* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1, 5-6, 8-12, 15-24, 26-27, 30-38* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's amendments to the claims filed May 30, 2008 and response to the election of species requirement filed 08/28/2008 have been entered. Claims 2-4, 7, 13-14, 25, 28 and 29 have been canceled, while claims 1, 26-27 and 30 have been amended. Applicants have also included new claims 31-38.

Claims 1, 5-6, 8-12, 15-24, 26-27, 30-38 are pending in this application.

Election/Restrictions

Applicant's election with traverse of the invention of group IV (27) filed October 24, 2005 was acknowledged. Applicant's argument of examining method for gene knock down in a vertebrate (group 1) with elected group were found persuasive, therefore invention of group I and IV directed to vertebrate and method of gene knock down in a vertebrate were rejoined for the examination purposes.

It was noted that, in response to office action dated 11/30/2007, applicants had added new claims 31-38 to include patentably distinct shRNA sequence set forth in SEQ ID NO 19-220. The sequences were independent or distinct because claims to the different shRNA for gene recite the mutually exclusive characteristics of such shRNA sequence. These shRNA sequences were not previously presented. Accordingly, a new election of species was required in view of newly added claims 31-38. In response to the secondary restriction requirement dated 08/28/2008, applicants' applicants have elected SEQ ID NO: 23 generic to claim 31 with traverse. Applicants' argument that it would not be undue search burden to examine transgenic mouse and method of plurality of different gene knockdown was found not persuasive. As stated before, In addition, these sequences have distinct physical and chemical structure and therefore these sequences would not be coextensive in patent and non patent literature (also see MPEP 803.04 and

Examination of Patent Applications Containing Nucleotide Sequences, 1192 O.G. 68 (November 19, 1996). The restriction is still deemed proper and therefore made FINAL.

Claims 1, 5-6, 8-12, 15-24, 26-27, 30-38 are under current examination.

Priority

It is noted that instant application claims benefit from application number 60/485,969 07/10/2003 that claims benefit of 60/467,814 filed on 05/02/2003, which claims benefit from 60/420,476 filed on 10/22/2002. Upon review of the disclosure of the prior-filed application, '969, '814 and '476 fails to provide descriptive support for instant claims 31-38 generic for elected species of SEQ ID NO: 23. There is not adequate support or enablement for claims 31-38 in the manner provided by the first paragraph of 35 U.S.C. 112 in any of these applications. In case, if applicants have evidence to support otherwise, applicants are invited to indicate page and line number for the written support as recited in claims 31-38 generic for SEQ ID NO: 23 of the instant application. Therefore, the effective filing date for instant claims 31-38 that is generic for SEQ ID NO: 23 is 10/15/2003.

Withdrawn-Claim Objections

The objection to claim 1 is withdrawn in view amendments to claim 1 that now recites homologous.

New-Claim Objections

Claim 1 is objected to because of the following informalities: the recitation of "the nonhuman vertebrate" in line 4 should be changed to "mouse" commensurate with the scope of preamble and rest of the claims. Appropriate correction is required.

Maintained- Claim Rejections- 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1, 5-6, 8-12, 15-24, 26 and 27 remain rejected under 35 U.S.C. 112, first paragraph, and newly added claims 37-38 are also rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of gene knockdown in a mouse genome at the *rosa26* locus, said method comprising introducing by homologous recombination into the Rosa26 locus a reporter construct comprising a *shRNA* into a mouse embryonic stem cell , wherein said *shRNA* and reporter constructs comprises a gene encoding Renilla (Rluc) and luciferase (Fluc) along with an adenovirus splice acceptor sequence and polyadenylation signal placed downstream of the endogenous promoter of rosa26, and Fluc specific shRNA expressed under the control of H1 and U6 promoter and terminated by five thymidine; and microinjecting said mouse embryonic stem cell into mouse diploid blastocysts; and implanting the blastocysts comprising the mouse embryonic stem cell into pseudo pregnant mouse; allowing the resulting pregnant mouse to deliver viable offspring and a transgenic mouse produced by said method, wherein said transgenic mouse exhibits ~90% reduced luciferase activity in liver, heart, brain and muscle, does not reasonably provide enablement for the transgenic mouse whose genome comprises a construct comprising SEQ ID NO: 23 and a method of gene knockdown to generate a transgenic mouse whose genome comprises a construct comprising SEQ ID NP: 23 (ras-gap). The specification does not enable any person skilled in the art to which it pertains, or with which it is most

nearly connected, to make and use the invention commensurate in scope with these claims.

Applicants' amendments and arguments filed 05/3/2008 have been fully considered but are not persuasive. Applicants argue that the examples are meant to be just that--exemplary of the broader disclosure, all of which must be taken into consideration. Applicants assert that the fact that a large number of shRNA sequences were known in the prior art that persons skilled in the art would expect would be operable and a large number of these are expressly set forth in the instant specification, for example, SEQ ID NOS.: 19-220, and, moreover, the specification teaches these work (see page 9 of the argument). Applicants also argue that none of the rejected claims require any particular degree of attenuation or any length of time in which attenuation is achieved, therefore this position is irrelevant. Applicants also argue that a gene knockdown be achieved. Accordingly, any assertion which relate to inoperable embodiments that are not claimed, are irrelevant.

In response, it is noted that claims are directed to a method of gene knock down in a mouse or a transgenic mouse whose genome comprises a construct comprising shRNA. Furthermore, newly added claims limit the generic claims to include a construct comprising SEQ ID NO: 23 (ras-gap). Contrary to applicants' assertion that method of gene knock down with any shRNA sequence or with SEQ ID NO: 19-220 would be operable, it is emphasized that unpredictability in achieving specific gene knockdown associated with a specific phenotype for the claimed method and transgenic mouse would require one of ordinary skill in the art to perform undue experimentation to make and use the claimed method and product without reasonable expectation of success. It is noted that claims embrace stably integrating by homologous recombination an expression vector into a polymerase II dependent locus wherein the expression vector comprises any shRNA under control of any ubiquitous promoters and homologous sequence that integrates

through homologous recombination at polymerase II dependent locus in the genome of the mouse. The guidance provided in the specification is limited to a method to gene suppression of Renilla (Rluc) and luciferase. The specification teaches a method of shRNA within the vector of the invention comprises at least one DNA segment A-B-C wherein A is a 15 to 35, preferably 19 to 29 bp DNA sequence being at least 95%, preferably 100% complementary to the gene to be knocked down (e.g. firefly luciferase, p53, etc.); B is a spacer DNA sequence having 5 to 9 bp forming the loop of the expressed RNA hair pin molecule, and C is a 15 to 35, preferably 19 to 29 bp DNA sequence being at least 85% complementary to the sequence A (see para 40-44 of the specification). It is emphasized that as recited independent claim embraces a DNA segment of any length, however the specification does not support the use of the large DNA segment gene in the expression construct. It is in this context, Examiner has cited prior and post filing art to show the specific nucleotide length requirements for RNAi to be effective to achieve specific phenotype resulting from the claimed method or mouse. Elbashir et al (Genes Dev. 2001 15; 15(2):188-200, IDS) teaches that 21 and 22 mer act as act as guide RNAs for sequence specific mRNA degradation and therefore act most effectively in RNAi. Elbashir et al also describes that 30 bp dsRNA are ineffectively processed to 21-22nt RNA suggesting ineffective gene silencing by larger nucleotide sequence (page188 col. 2, para 2). The specification further describes stably integrating, preferably at a polymerase II dependent locus of the living mouse. The specification further discloses that polymerase II dependent loci include Rosa26 locus, collagen, RNA polymerase, actin and HPRT. Prior to instant invention, the unpredictability of attenuating /inhibiting expression of a target gene in cell by RNAi is evident in prior and post filing art. While it is recognized, that introduction of dsRNA that is targeted to a specific gene may result in attenuation /inhibition of the targeted gene, the degree of attenuation and length of the time attenuation is achieved in not predictable. Caplen et al (Gene 2000, vol. 252, 95-105, art of record) provide evidence of the

unpredictability of dsRNA attenuation /inhibition of targeted gene in vertebrate cell *in vitro.* Transient transfection of dsRNA to the βgal transgene into 293 and BHK31 cells resulted in either no effect or a non-specific decrease in gene expression (pp102; Figure 7 A and B). Prior to instant invention, homologous recombination in embryonic stem cells has been widely used to produce mice carrying a single copy of the transgene integrated into a predetermined site including polymerase II dependent locus of the genome (Bronson et al., Proc. Natl. Acad. Sci. USA, 93(17:9067-72 (1996) art of record). However, art also teaches stably integrating at any polymerase II dependent locus would not necessarily yield predictable gene knock down. This is evident from expression of the low expression of the transgene. For instance, Hatada et al. demonstrated that the HPRT locus suppresses the activity of both, the haptoglobin gene promoter as well as the herpes simplex thymidine kinase promoter in several tissues of mice (Hatada et al., J. Biol., Chem., 274(2):948-55, 1999, IDS). Likewise, a human eNOS promoter-LacZ reporter gene placed in the Hprt locus was found to be inactive in hepatic vessels that otherwise express the endogenous eNOS gene (Guillot et al., Physiol. Genomics, Mar. 13, (2):77-83, 2000). Thus, in view of foregoing it is apparent that the several factors confounded the method for gene knockdown and integration of expression vector comprising shRNA into any polymerase II dependent locus the genome of the mouse that would not necessarily result in gene knockdown commensurate with full scope of the claims to make use of the invention. Moreover, there is no guidance teaching to how to use the claimed animals or a general method of gene knockdown using DNA segment of any length and integration of expression vector comprising shRNA into any polymerase II dependent locus the genome of the mouse. Thus, the specification fails to provide an enabling disclosure for using the claimed mouse and method of making the transgenic mouse as claimed, for reasons of record. It was indicated that the unpredictability of a particular art area may alone provide reasonable doubt as to the accuracy of the broad statement made in support of

enablement of claims. See *Ex parte Singh, 17 USPQ2d 1714 (BPAI 1991).* It is also well established in case law that the specification must teach those of skill in the art how to make and how to use the invention as broadly claimed. *In re Goodman, 29 USPQ2d at 2013 (Fed. Cir. 1994),* citing *In re Vaeck, 20 USPQ2d at 1445 (Fed. Cir. 1991).* An artisan would have to perform undue experimentation without reasonable expectation of success in order to make and use the invention commensurate with full scope of the claims.

Applicant's amendments to the claim limiting the method and product directed to the transgenic mouse overcome the rejection pertaining to the issue of embryonic stem cells in claims 1, 5-6, 8-12, 15-25, and 27 is hereby withdrawn. However, the rejection is maintained for claim 26 that read on a method of gene knock down of claim 1, in a nonhuman vertebrate comprising integrating the expression vector into ES cells of the nonhuman vertebrate. The art at the time of filing held that transgenic technology using ES cell was not predictable for any species other than mouse. Since the specification discloses using mouse ES cells to produce transgenic mice via homologous recombination of targeting vectors in the ES cells, ES cells from various species of fish would be required as recited in the claims. However, prior and post filing art teaches that germline competent embryonic stem cells are not available in any other mammalian species other then mouse (Denning et al., Reproduction 126:1-11, 2003, Hochepied et al Stem Cells, 2004, 22, 441-447; abstract). It is noted that several years after filing of instant application, Hong et al (Methods Mol Biol. 2006;329:3-16) provided guidance with respect to obtaining medaka ES cells, however, it is generally known in the art that homologous recombination of a targeting construct had not been attempted with ES like cells available prior to filing of this application. In fact a recent report Alvarez et al (Mar Biotechnol . 2007 Mar-Apr;9(2):117-27) acknowledges the slow progress made in recent years and asserts that "there are promising achievements in homologous recombination and alternative avenues yet to be explored that can

bring ES technology in fish to fruition" (abstract). This clearly shows that targeting gene by homologous recombination using fish ES cells was still evolving at the time of filing of this application. In the instant case, neither specification nor prior art provided any guidance with respect to targeting a construct for stably integrating by homologous recombination at a polymerase II dependent locus a construct comprising shRNA under the control of a promoter in any species of fish for the purposes of gene knockdown. Therefore, at the time of filing of this application, method of gene knockdown in could not have been accomplished for any species of fish other than mouse. The specification does not teach how to make knock down nonhuman vertebrate by shRNA for any other species other than mice or correlate making mice to making knockout vertebrate of any species. Therefore, the claims should be limited to mouse and method for gene knockdown in mouse as discussed in the previous office actions. An artisan would have to perform undue experimentation for effective reduction to practice without reasonable expectation of success, especially in view of the evidence that is contrary to the instant claims.

With respect to claims 27 and newly added claim 38, contrary to applicants' assertion that Examiner has raised the same issue directed to fish, it is noted that the issue was discussed with respect to mouse and fish both. It was emphasized that as recited instant claims do not require any specific phenotype for the claimed transgenic mouse. It is in this context it was stated that disclosed phenotype could not be correlated to resulting phenotype in any other species of vertebrate. The state of the art at the time of filing uses the unpredictability of obtaining transgenic animals with a specific phenotype as summarized by the references of Keri et al., (Proc Natl Acad Sci U S A. 2000; 97(1):383-7) show that elevated levels of lutenizing hormone in transgenic can result in different reproductive system abnormalities including ovarian tumors. Similarly, Carmell et al failed to produce any distinct phenotype, while shRNA, constructs directed against seven known targets were introduced via standard trasngenesis in mouse (Carmell MA Nat Struct Biol. 2003;

10(2): 91-92, art of record). Thus it is clear from the cited arts that at the time of filing, the resulting phenotype of a gene knockdown resulting from methods routinely used for integrating shRNA in the genome of mouse/nonhuman vertebrate was considered unpredictable. In the instant case, specification as filed does not provide any specific information about resulting phenotype of the nonhuman vertebrate or mouse. It is noted that the specification merely recites the luciferase activity in different organ, however it dose not provide any specific information for practicing the claimed invention commensurate with the full scope of the claim. Holschneider et al. (Int J Devl Neuroscience, 2000, 18: 615-618) state that single genes are often essential in a number of different physiological processes. Hence, deletion of an individual gene may prove so drastic or so widespread as to create an amalgam of phenotypes whose interpretation becomes confounded by the interaction of various new physiologic changes (pp 615). Holschneider et al discuss various factors that contribute to the resulting phenotype of transgenic mice, including compensatory system that may be activated to mask the resulting phenotype; these compensatory changes may be due to differential expression of another gene, which may be regulated by the downstream product of the deleted gene. The newly added claim 38 do not require any specific phenotype of resulting transgenic mouse and one of skilled in art would not know how to use a mouse whose genome comprises a construct comprising SEQ ID NO: 23. Given that the resulting phenotype of a knockdown mouse was considered unpredictable and it was confounded by multiple compensatory pathways. The specification does not teach any transgenic mouse comprising any knockdown of any level would result in expected phenotype. An Artisan of skill would need to perform further research upon the nonhuman vertebrate /mouse obtained by the process disclosed in the instant application in order to determine the correlation between the transgene knockdown and the observed phenotypes or effect. In absence of any specific teaching or arguments, an artisan of skill would have to perform undue

experimentation to make new invention in the field to make use of the invention. An artisan would have to perform undue experimentation to determine the appropriate elements that would specifically express genus of different genes or SEQ ID NO: 23 in the mouse showing expected phenotype. Absent of evidence to the contrary, it is not clear that resulting phenotype of a nonhuman vertebrate/mouse comprising genus of shRNA of known or unknown biological function particularly in view of unpredictably expressed in the art. An artisan would not know how to use resulting mouse made by the method described in the specification and therefore would require undue experimentation to determine how to use the resulting transgenic nonhuman vertebrate.

In view of lack of teaching or guidance provided by the specification with regard to an enabled method for gene knockdown in any vertebrate comprising a disruption in gene using any shRNA, construct comprising different constitutive or inducible promoter, and shRNA sequence and the lack of teaching or guidance provided by the specification to overcome the art recognized unpredictability of disruption of a particular gene, promoter and locus and the resulting phenotype and absence of any correlation between disruption and its phenotype, for the specific reason cited above in the office action. It would require undue experimentation for an Artisan to make and use the claimed invention and/or working examples demonstrating the same, such invention as claimed by the applicant is not enabled for the claimed inventions commensurate with the full scope of the claims.

Withdrawn-Claim Rejections- 35 USC § 112

Claims 1, 5-6, 8-12, 14-24 and 26 were rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. Applicants'

arguments filed 5/3/2008 has been fully considered and are persuauive. Therefore, rejection is hereby withdrawn.

Maintained-Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claims 1, 5-6, 8-10, 15-16, 18, 20-24, 26-27 and 30 are rejected under 35 U.S.C. 103(a) and newly added claims 31-34, 36-38 are also rejected under 35 U.S.C. 103(a) as being unpatentable over McCaffrey et al., (Nature, 2002 Vol. 418, 38-39) or Beach et al. (US patent Publication no. 2003/0084471, dated 5/1/2003, effective filing date 1/22/2002) and Bronson et al (Proc Natl Acad Sci U S A 1996; 93:9067–9072).

Claims 31, 36-38 are included in the rejection to the extent claims read on the limitation of claim 36 (shRluc and shFluc).

Applicants' arguments filed May 30, 2008 have been fully considered but are not persuasive. Applicants argue that claim 1 has been amended to require that a

gene knockdown is, in fact, achieved. Applicants assert that there is no reasonable expectation of success and a *prima facie* case of obviousness is not made out. Applicants assert that neither McCaffrey nor Beach discloses stably integrating a shRNA expression construct into a polymerase II dependent locus for any purpose, let alone for achieving gene knockdown. Examiner has taken the position that it was not predictable that stable integration of shRNA at a polymerase II dependent locus would be successful. The examples in the instant specification show gene knockdown is successfully achieved and the claims have now been amended to require that gene knockdown is successfully achieved.

In response, it is noted that had McCaffrey et al disclosed stable integration of a shRNA expression construct into a polymerase II dependent locus then this would have been an anticipation rejection and not an obviousness type rejection. Applicants should note that it is well established in case law that a reference must be considered not only for what it expressly teaches, but also for what it fairly suggests. In re Burkel, 201 USPQ 67 (CCPA 1979). Furthermore, in the determination of obviousness, the state of the art as well as the level of skill of those in the art is important factors to be considered. The teaching of the cited references must be viewed in light of these factors. It also appears that applicant is attempting to attack each reference individually. However, in a 103 rejection the references must be considered as a whole. In the instant case, claims are directed to a method of gene knockdown, an expression vector comprising a shRNA and a transgenic mouse whose genome comprises said expression vector. It is emphasized that claim 1 only recite one active method step of integrating by homologous recombination an expression vector. Contrary to applicants' assertion that claimed invention is limited by a specific phenotype, it is emphasized that recitation of achieving a reduction in the activity of gene product to any level is not specific phenotype. Additionally, the expression vector and transgenic mouse set forth in the independent claims do not distinguish to the expression vector and the resulting

mouse disclosed by the combination of cited references. In the instant case, McCaffrey/Beach both teach a method of gene knock down in a mouse by administering an expression vector comprising shRNA under the control of ubiquitous promoter that integrates in the mouse genome and achieve greater than 30% reduction is the expression of gene product. The deficiency of McCaffrey/Beach is cured by Bronson who describes problems associated with DNA incorporated into the mouse germ line using this method includes random integration and unpredictable copy numbers. Bronson emphasize that random integration often also presents profound effect on expression of the transgene resulting in altered phenotype of the mouse (see page 9067, col.1, para. 1). In fact, Bronson provided advantages of targeting a single copy of a transgenic sequence to a chosen location in the genome such as HPRT over random integration of construct. Bronson discloses many advantages of targeting at specific locus including the ability to control copy number, the ability to insert the transgene into regions of chromatin compatible with a desired developmental and tissue-specific expression. It is noted that Bronson et al emphasize that targeted transgenes provide a more efficient and informative means of securing and comparing the expression of various transgenic sequences than is available with current transgenic procedures. Bronson et al teach a method that uses homologous recombination in embryonic stem (ES) cells to generate mice having a single copy of a transgene integrated into a chosen location in the genome. Specifically, Bronson et al disclose a method wherein a single copy murine bcl-2 cDNA driven by either a chicken beta-actin promoter or a human beta-actin promoter has been inserted immediately 5' to the HRPT locus by a directly selectable homologous recombination event (see the abstract and figure 2).

It is noted that obviousness does not require absolute predictability of success; for obviousness under 35 U.S.C. § 103, all that is required is a reasonable expectation of success. See In re O'Farrell, 7 USPQ2d 1673 (CAFC 1988). In the instant case, claims merely require achieving a reduction in the activity of a gene

product. It is emphasized that prior art taught both the expression construct and method of gene knockdown in mouse using said expression vector comprising small-hairpin RNAs (shRNAs) that is expressed *in vivo* from DNA templates using RNA polymerase III promoters inhibited the luciferase expression by up to 98% (pp38, Figure 1 C-D and pp39 2nd paragraph). It is noted that McCaffrey taught shRNA that comprises at least one DNA segment A-B-C wherein A is a 15 to 29 bp DNA sequence with at least 100% complementarily to the gene to be knocked down; B is a spacer DNA sequence having 5 to 9 bp forming the loop of the expressed RNA hairpin molecule, and C is a 19 to 329 bp DNA sequence and further comprises a poly A sequence meeting the limitation of claims 20-24 (see the supplementary information). Thus, expression construct disclosed by McCaffre/Beach is structurally similar to one claimed in the instant application. Furthermore, both McCaffre/Beach taught a method of gene knockdown using the construct comprising shRNA by random integration of the construct. Given that method to stably integrate expression cassette at a chosen site in the genome was know in prior art. It would have been *prima facie* obvious for one of ordinary skill in the art to express the shRNA expression cassettes that are flanked by homology regions for the endogenous loci such as hprt. It is further noted that the method that uses homologous recombination in embryonic stem (ES) cells to generate mouse having a single copy of a transgene integrated into a chosen location in the genome and advantage for targeted transgenes to attain more efficient expression was known in prior art, it would have been *prima facie* obvious for one of ordinary skill in the art to combine the teaching with reasonable expectation of success. Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Claims 1, 5, 27 and 30 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Beach et al. (US patent Publication no. 2003/0084471, dated

5/1/2003, effective filing date 1/22/2002) or McCaffrey et al., (Nature, 2002 Vol. 418, 38-39, art of record); Bronson et al (Proc Natl Acad Sci U S A 1996; 93:9067–9072, art of record) as applied to claims 1, 5-6, 8-10, 15-16, 18, 20-24, 26-27 and 30-34, 36-38 above, and further in view of Soriano et al (US patent 6,461,864, October 8, 2002, art of record).

Applicants' arguments filed May 30, 2008 have been fully considered but are not persuasive. Applicants argue that the Examiner relies on Soriano to show expression from the rosa26 locus. However, such expression is still not of a shRNA or for the purpose of achieving gene knockdown. Accordingly, the combination of McCaffrey, Beach, Bronson and Soriano fails to teach the achievement of gene knockdown by incorporation of shRNA at a polymerase II dependent locus with a reasonable expectation of achieving successful gene knockdown.

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986).For the purpose of combining references, those references need not explicitly suggest combining teachings, much less specific references. In re Nilssen, 7 USPQ2d 1500 (Fed. Cir. 1988). As stated before combination of Beach /McCaffrey and Bronson taught a method of stably integrate by homologous recombination an shRNA construct under the control of a promoter in polymerase II dependent locus (HPRT), but differed from claimed invention by not disclosing stably integrating into other polymerase II dependent locus such as Rosa26. The deficiency is cured by Soriano et al who provided guidance with respect to ubiquitously expressed gene loci for use include Rosa 26, rosa5 and others (see col. 3, lines 49-54). Soriano et al teach methods and vector constructs for the production of genetically engineered non-human animals, which ubiquitously express a heterologous DNA segment in Rosa 26 locus (abstract and claim 1).

It would have been obvious for one of ordinary skill in the art at the time of invention to modify the method of gene knock down disclosed by McCaffrey/Beach to include shRNA expression cassettes that are flanked by homology regions for the polymerase II dependent locus (rosa26) by homologous recombination in ES cells to generate mouse having a single-copy of a transgene inserted at a chosen site in the genome. Bronson provided guidance by emphasizing that the use of a chosen site for a single copy of a transgene avoids many of the problems associated with randomly inserted transgenes (see supra and page 9072, col. 1, last paragraph). The reference of Soriano provided guidance with respect to ubiquitously expressed gene loci for use include Rosa 26, rosa5 and others (see col. 3, lines 49-54). It would have been obvious for one of ordinary skill in the art to use the method of gene knock down in mouse by modifying the shRNA expression cassettes under the control of the CMV/H1 or U6 promoter as disclosed by McCaffrey/Beach and then flanking by homology regions for the Rosa26 locus to stably integrate expression cassette comprising an shRNA under control of ubiquitous promoter into a specific locus such as HPRT/rosa26 as discussed by Bronson with reasonable expectation of achieving predictable result to more efficiently suppress the transgene expression. It is noted that several polymerase II dependent loci were known at the time of filing of this application and it would have required only routine experimentation to flank expression cassettes comprising shRNA under the control of a promoter with the homology regions of other polymerase II dependent locus (See MPEP2144.04). One who would practiced the invention would have had reasonable expectation of success because McCaffrey/Beach had already described a method for gene knockdown in a mice by random integration of the construct, and it would have been routine to use express transgene in a chosen site to avoid many of the problems associated with randomly inserted transgenes as evidenced from the teaching of Bronson. Thus, it would have only required routine experimentation to modify the expression construct that are flanked by homology regions for the

polymerase II dependent locus as disclosed by Bronson. One of ordinary skill in the art would have been studied Bronson to combine the teaching of Beach/ McCaffrey and Soriano because a method of gene knockdown in a mouse comprising a shRNA construct under control of a ubiquitous promoter into a specific polymerase II dependent locus that included hprt, rosa26 or any other endogenous loci would have provided stable and sustained expression of short hairpin resulting in gene knockdown. Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Claims 11-12, 17 and 19 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Beach et al. (US patent Publication no. 2003/0084471, dated 5/1/2003, effective filing date 1/22/2002, art of record) or McCaffrey et al., (Nature, 2002 Vol. 418, 38-39, art of record); Bronson et al (Proc Natl Acad Sci U S A 1996; 93:9067–9072, art of record) and Soriano et al (US patent 6,461,864, October 8, 2002) as applied to claims 1, 5-6, 8-10, 15-16, 18, 20-24, 26-27 and 30-34, 36-38 above, and further in view of Ohkawa et al (Hum Gene Ther. 2000; 11 (4): 577-85; IDS).

Applicants' arguments filed May 30, 2008 have been fully considered but are not persuasive. Applicant's arguments all rely on the references of McCaffrey/ Beach, Bronson and Soriano that has been previously discussed. In absence of any other arguments rejection is maintained for the reasons of record.

New-Claim Rejections-Necessitated by amendments- 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject

matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 31-35, 37-38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Beach et al. (US. 2003/0084471, dated 5/1/2003, effective date 1/22/2002) or McCaffrey et al., (Nature, 2002 Vol. 418, 38-39, art of record); Bronson et al (Proc Natl Acad Sci U S A 1996; 93:9067–9072, art of record), Soriano et al (US patent 6,461,864, , 2002, art of record) and Kunath et al., (Nature Biotechnology, 21: 559-561, 2003, IDS).

The teaching of Beach et al or McCaffrey, Bronson and Soriano et al have been discussed above and relied in same manner here. Although combination of Beach /McCaffrey, Bronson and Soriano et al taught a method of stably integrate by homologous recombination a shRNA construct under the control of a promoter in polymerase II dependent locus but differed from claimed invention by not disclosing shRNA being SEQ ID NO: 23.

However, prior to instant invention, with respect to claim 31, 35, Kunath et al teach a construct comprising DNA encoding the human H1 RNA pol III promoter and a RasGAP shRNA sequence (SEQ ID NO: 23, 100% sequence homology) (see page 561, col. 1, para. 3). Regarding claims 37 and 38, Kunath et al teach a method of gene knockdown by providing the expression vector comprising SEQ ID NO: 23 that is integrated in the genome of ES cells that resulted in inhibition of RasGAP protein (see figure 1 and 2).

It would have been obvious for one of ordinary skill in the art at the time of invention to modify the method of gene knock down disclosed by McCaffrey/ Beach to include the shRNA expression cassettes that are flanked by homology regions for the polymerase II dependent locus as disclosed by Bronson and Soriano to stably integrate by homologous recombination in ES cells to generate mouse having a single-copy of a transgene inserted at a chosen site in the genome. Bronson provided guidance by emphasizing that the use of a chosen site for a single copy of a

transgene avoids many of the problems associated with randomly inserted transgenes (see page 9072, col. 1, last paragraph). It would have been *prima facie* obvious for one of ordinary skill in the art to make transgenic mouse that comprises stably integrated expression vector into a specific locus by homologous recombination as discussed by Bronson and Soriano in order to more efficiently suppress the transgene expression for sustained period. One who would practiced the invention would have had reasonable expectation of success because Kunath, McCaffrey/Beach et al had already described a method for gene knockdown in a mice by transiently as well as stably expressing shRNA construct and it would have only required routine experimentation to modify the expression construct that are flanked by homology regions for the polymerase II dependent locus as disclosed by Bronson and Soriano. One of ordinary skill in the art would have combined the teaching because a method of gene knockdown in a mouse comprising a shRNA construct under control of a ubiquitous promoter into a specific polymerase II dependent locus would have provided stable and sustained expression of ras-gus (SEQ ID NO: 23) resulting in gene knockdown.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 1, 5-6, 8-12, 15-24, 26-27, 30 remain provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claim14-16, 18-44 of copending Application No. 10/531,347 for the reasons of record. Applicants have indicated that they would consider filing terminal disclaimer upon notice of allowable subject matter in this application.

Conclusion

No Claims allowed.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Gossen et al (Proc Natl Acad Sci U S A. 1992 Jun 15;89(12):5547-51).

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL.** See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will

the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ANOOP SINGH whose telephone number is (571)272-3306. The examiner can normally be reached on 9:00AM-5:30PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras can be reached on (571) 272- 4517. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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